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351. (Twice amended) A method according to Claim 329 wherein said targeting ligand is a peptide comprising a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1).

REMARKS

Reconsideration of the present application in view of the above amendments and following remarks is requested respectfully.

Status of the Claims

Claims 100, 102, 103, 127, 194 to 200, 203, 210 to 228, 294 to 300, 303, 310 to 329,331 to 337, and 347 to 356 are pending in the application. Claims 113, 115, 122, 124, 229 to 238, 245 to 248, 255 to 270, 277 to 280, 287 to 292, and 357 to 411 have been canceled, without prejudice. No claims have been added. Claims 100, 127, and 351 have been amended.

Claims 100 and 127 have been amended to define targeted lipid vesicles that comprise a conjugate that comprises a lipid, a linking group, and a targeting ligand, wherein the linking group is a hydrophilic polymer that is covalently bound to both the linking group and the targeting ligand. Support for the claimed conjugate may be found in the specification, for example, at page 80, line 16, to page 92, line 15. The term "conjugate" is specifically recited in the specification, for example, at page 86, line 18. Claim 100 has further been amended to recite that the hydrophilic polymer is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof. Support for the claimed hydrophilic polymers may be found, for example, at page 86, lines 4 to 15.

The amendment to Claim 351 clarifies that the claimed targeting ligands are peptides which comprise the recited amino acid sequences. Support for this language may be found in the specification, for example, at page 67, lines 7 to 9, or page 73, lines 7 to 10. It is

believed that this claim amendment, in conjunction with the cancellation of claims, fully addresses the pending rejection under 35 U.S.C. § 112, second paragraph.

Applicants thank the Examiner for indicating that the present application has an effective filing date of May 1, 1996. Applicant respectfully asserts that the amended claims are also fully supported by U.S. Application Serial No. 08/640,464, filed May 1, 1996.

Summary of the Invention

The invention defined by the pending claims is directed to formulations for diagnostic or therapeutic use that comprise targeted lipid vesicles having encapsulated therein a gas selected from the group consisting of perfluorocarbons and sulfur hexafluoride, and methods for therapeutic delivery *in vivo* that comprise administering such formulations. Unlike the art cited by the Examiner, Claims 100 and 127, the only two pending independent claims, both importantly recite that the targeted lipid vesicles comprise a *conjugate that comprises a lipid, a linking group, and a targeting ligand that is covalently bound to both said lipid and the targeting ligand.* Moreover, the linking group is a hydrophilic polymer selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and the targeting ligand is one which targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor.

Rejection under Section 102(e)

Claims 100, 102, 127, 194, 203, 294, 303, and 320 to 338 stand rejected under 35 U.S.C. 102(e) as being anticipated by Lanza et al., U.S. Patent No. 5,989,520 ("Lanza").[1]

[1] In the interest of brevity, Applicants will not reiterate the arguments previously presented as to why Lanza is not proper prior art to the instant application. Applicants again

Applicants respectfully submit that the previously pending claims define over Lanza, for the reasons, for example, presented in the amendment filed March 7, 2002. Nonetheless, in the interest of advancing prosecution of this application, Applicants have amended the claims to even further define over Lanza

Lanza is generally directed to a method for ligand-based binding of lipid encapsulated particles to molecular epitopes on a surface comprising *sequentially* administering (a) a site-specific ligand activated with a biotin activating agent; (b) an avidin activating agent; and (c) lipid encapsulated particles activated with a biotin activating agent, whereby the ligand is conjugated to the particles through an avidin-biotin interaction so that the resulting conjugate is bound to the molecular epitopes. *See* Lanza abstract. Perfluorcarbon emulsions may be encapsulated with the lipid particles, and the emulsions may generate gaseous vapors. *See* col. 6, lines 1 to 24, and col. 7, lines 1 to 6. Lanza further describes both diagnostic and therapeutic applications for this system. *See* col. 7, lines 41 to col. 8, line 13.

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respectfully assert, however, for the record, that it is improper for the Examiner to refer to any disclosure in Lanza that was not part of an application for patent filed before the effective filing date of the instant application. In specific, Applicants note that Example 18 of Lanza is not present in U.S. Application Serial No. 08/488,743 (now U.S. Patent No. 5,690,907), and may not properly be relied on by the Examiner in rejecting the instant application. The discussion in Applicants' Response dated March 7, 2002, regarding the unavailability of Lanza as prior art to the present claims is incorporated herein by reference.

Lanza Does Not Anticipate Applicant's Claimed Invention

Applicants claims distinguish over Lanza, *inter alia*, by defining targeted lipid vesicles that comprise a *conjugate that comprises a lipid, a linking group, and a targeting ligand*, wherein the linking group is a hydrophilic polymer that is covalently bound to both the lipid and the targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof. Moreover, Lanza does not teach lipid vesicles that encapsulate a gas selected from the group consisting of perfluorocarbons and sulfur hexafluoride.

Lanza does not describe Applicants' targeted lipid vesicles. Lanza is clearly and unequivocally directed to the use of an avidin/biotin reaction to bind the targeting ligand to the lipid encapsulated particle (or liposome). Thus Lanza does not teach or suggest the formulations defined by Applicants claims. Instead, Lanza teaches that lipid encapsulated particles may be biotinylated, *i.e.* may contain a conjugate that comprises lipid covalently bound to biotin. This is not the conjugate defined in applicants claims. Lanza also describes a targeting ligand bound to biotin, *i.e.* a conjugate that comprises a targeting ligand covalently bound to biotin. This entity also is not the conjugate defined in applicants claims. Ultimately, these entities may become bound together in the body of a patient through a biotin/avidin interaction, *i.e.* may form a conjugate that comprises lipid bound to targeting ligand through a biotin/avidin complex. *See* Lanza, col. 2, line 64 to col. 3, line 5. Even this final entity, formed in the body of a patient, is not the conjugate of Applicants claims, that comprises a lipid covalently bound to a hydrophilic polymer linking group, that is in turn covalently bound to a targeting ligand.

Moreover, although Lanza teaches the encapsulation of perfluorocarbon emulsions, such as perfluorotributylamine, perfluorodichloroctane, and the like, and suggests that vapors may be evolved from these dense liquids, these compounds are neither true

perfluorocarbons, nor true perfluorocarbon *gases*. Lanza thus does not anticipate Applicants' claimed invention for this reason, if for no other.

Moreover, Lanza's tri-phasic, sequential administration procedure (*see id.*) is clearly completely different than the methods of the present invention, which mention no such tri-phasic administration. As discussed above, in Lanza's method, three different compositions are administered to the patient *separately and sequentially*: first, the patient is administered a site-specific ligand activated with a biotin activating agent; second, the patient is administered an avidin activating agent; and third, the patient is administered lipid encapsulated particles activated with a biotin activating agent. Thus, the patient is ***never*** administered a vesicle composition that comprises targeted lipid vesicles that comprise a conjugate comprising a lipid covalently bound to a hydrophilic polymer linking group, that is in turn covalently bound to a targeting ligand, wherein the targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor, as recited in Applicants' claims.

In view of the foregoing, Applicants respectfully submit that it is absolutely clear that Lanza does not disclose or suggest either the compositions or methods defined by Applicants' claims, either as presented previously, or as amended herein. Accordingly, Applicants respectfully request that the rejection under Section 102(e) be withdrawn.

Rejection under Section 103(a)

All pending claims stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Porter, U.S. Patent No. 5,648,098 ("Porter"), in view of Lanza and Konigsberg et al., U.S. Patent No. 5,258,499 ("Konigsberg") or Trubetskoy et al., Biochemica et Biophysica Acta 1131 (1992) 311-313 ("Trubetskoy), and Ginsberg, U.S. Patent No. 5,656,442 ("Ginsberg") and Siegel et al., U.S. Patent No. 5,695,460 ("Siegel"). Applicants respectfully traverse this rejection, on

the grounds that the references, in any proper combination, fail to teach of suggest the formulations and methods of the present invention.

Porter, the primary reference relied upon in the Section 103 rejection, describes a method of treating thrombosis comprising administering a composition of "perfluorocarbon enhanced sonicated dextrose albumin microbubbles" (referred to hereinafter, as in Porter, as "PESDA microbubbles") and applying ultrasound to the site of the thrombus. *See e.g.*, col. 6, Example 1. It is conceded in the Office Action that Porter does not teach the use of a lipid vesicle. Of course, since Porter discloses neither lipid vesicles nor targeting ligands, Porter completely fails to suggest that a targeting ligand may be covalently bound to the lipid via a hydrophilic polymer linking group. It is clear, therefore, that Porter in no way teaches, discloses or suggests the formulations defined by Applicants' claims. Moreover, Porter does not describe *any* methods for the therapeutic delivery *in vivo* of a bioactive agent, since Porter's pharmaceutical compositions do not contain any bioactive agent (*see, e.g.*, col. 2, lines 21 to 26).

The deficiencies of Porter are said in the Office Action to be overcome by combination with the other cited references. The disclosure of Lanza is discussed above. It is said in the Office Action that Lanza "is primarily used to show that liposomes containing perfluorinated emulsions provide improved targeting specificity when they are attached to a targeting ligand." Porter is not directed to liposomes, however, and while Lanza may show increased target specificity, this increased specificity is said to be for the purpose of *identifying sites* within the patient, not for the purpose of *lysing thrombi*, which is the purpose to which Porter is directed. Thus, there is no suggestion in either of the references which would motivate one skilled in the art to modify the PESDA microbubbles used by Porter by using gas-filled liposomes that bear a targeting ligand. In fact, Lanza emphasizes the desirability of using vesicles at extremely low blood concentration levels. *See* Lanza col. 7, lines 29 to 32 and 48 to 52 ("the background contrast from lipid encapsulated particles in the blood is minimal").

Applicants respectfully submit that the skilled artisan would have no reason to believe that such low blood levels of vesicles would be sufficient to achieve the thrombolysis that is the subject of Porter. The skilled artisan would therefore have no motivation to modify Porter's vesicles using the methods described by Lanza, because there would be no reasonable expectation that such a modification would succeed. Applicants respectfully submit that the combination of the two references, as done in the Office Action, is improper for this reason also.

Moreover, even if Porter is combined with Lanza, the deficiencies of Lanza which were discussed with regard to the Section 102 rejection are still evident. Lanza does not teach or suggest methods involving a formulation comprising lipid vesicles that comprise a conjugate according to Applicants' claims: to the contrary, Lanza describes a tri-phasic administration, in which the disclosed liposomes and targeting ligands are administered ***separately***. According to Lanza, the targeting ligand and the lipid vesicles may become bound together, *in vivo*, via an avidin/biotin complex. Thus, even if Lanza and Porter are improperly combined, as in the Office Action, one still fails to arrive at the methods and formulations defined by Applicants' claims.

These deficiencies are not remedied by further combination with Konigsberg. Konigsberg describes liposomes that have ligands covalently attached to the surface by coupling agents (linker molecules) such as SATA or SPDP, but contains no teaching or suggestion that a hydrophilic polymer may be used for this purpose (*see, e.g.*, col. 4, lines 1 to 46). Similarly, although Trubetskoy may well teach the preparation of targeted cationic liposomes via an ionic bridge between cationic liposomes and a targeting moiety, the targeting ligand is neither *covalently linked*, nor is it linked *via a hydrophilic polymer linking group*, as recited in Applicants' claims. Ginsberg, which describes the KQAGDV targeting ligand, fails to teach or suggest linking of same to a gas-filled lipid vesicle, and Siegel, which is devoid of any teaching or suggestion regarding the use of targeting ligands, also fails to overcome the deficiencies of the previously discussed references.

Any proper combination of these references does not, therefore, teach or suggest the methods and compositions defined by Applicant's claims. None of the references teach the use of hydrophilic polymer linking groups for covalently binding targeting ligands to lipid vesicles. Accordingly, Applicants respectfully request that the rejection under Section 103 be reconsidered and withdrawn.

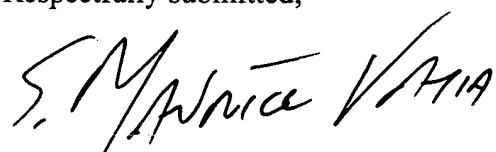
CONCLUSION

Applicants believe that the foregoing constitutes a full and complete response to the Office Action of record. Accordingly, an early and favorable allowance of all of pending Claims 100, 102, 103, 127, 194 to 200, 203, 210 to 228, 294 to 300, 303, 310 to 329,331 to 337, and 347 to 356 is requested respectfully.

In the event that the Examiner is not persuaded that the claims as amended herein are allowable, Applicants respectfully request that the Examiner contact Applicants' undersigned representative to arrange for a telephonic interview to discuss the application further.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**" Also attached is a copy of the claims Applicants believe to be pending after entry of the amendment, as requested by the Examiner.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 113, 115, 122, 124, 229 to 238, 245 to 248, 255 to 270, 277 to 280, 287 to 292, and 357 to 411 have been canceled, without prejudice.

Claims 100, 127, and 351 have been amended as follows:

100. (Twice amended) A formulation for therapeutic or diagnostic use comprising[, in combination with a bioactive agent] targeted lipid vesicles [encapsulating a fluorinated gas and bearing a targeting ligand, wherein said targeting ligand is covalently bound to said lipid vesicles via hydrophilic polymer linking groups] having encapsulated therein a gas selected from the group consisting of perfluorocarbons and sulfur hexafluoride, said targeted lipid vesicles comprising a conjugate that comprises a lipid, a linking group, and a targeting ligand, wherein said linking group is a hydrophilic polymer that is covalently bound to both said lipid and said targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and wherein said targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor [and said fluorinated gas is selected from the group consisting of perfluorocarbons and sulfur hexafluoride].

127. (Twice amended) A method for the therapeutic delivery *in vivo* of a bioactive agent comprising administering to a patient a therapeutically effective amount of a

formulation which comprises, in combination with a bioactive agent, targeted lipid vesicles [encapsulating a fluorinated gas and bearing a targeting ligand, wherein said targeting ligand is covalently bound to said lipid vesicles via hydrophilic polymer linking groups] having encapsulated therein a gas selected from the group consisting of perfluorocarbons and sulfur hexafluoride, said targeted lipid vesicles comprising a conjugate that comprises a lipid, a linking group, and a targeting ligand, wherein said linking group is a hydrophilic polymer that is covalently bound to both said lipid and said targeting ligand, and is selected from the group consisting of polyethylene glycol (PEG), polypropylene glycol, polyvinylalcohol, polyvinylpyrrolidone, and copolymers thereof, and wherein said targeting ligand targets cells or receptors selected from the group consisting of myocardial cells, endothelial cells, epithelial cells, tumor cells and the glycoprotein GPIIbIIIa receptor [and said fluorinated gas is selected from the group consisting of perfluorocarbons and sulfur hexafluoride].

351. (Twice amended) A method according to Claim 329 wherein said targeting ligand [comprises] is a peptide comprising a sequence selected from the group consisting of Arg-Gly-Asp and Lys-Gln-Ala-Gly-Asp-Val (SEQ ID NO 1).